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Fibroblast cell interactions with human melanoma cells affect tumor cell growth as a function of tumor progression

(metastasis/growth factors/tumor–host relationship)

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ABSTRACT **It is known from a variety of experimental systems that the ability of tumor cells to grow locally and metastasize can be affected by the presence of adjacent normal tissues and cells, particularly mesenchymally derived stromal cells such as fibroblasts. However, the comparative influence of such normal cell–tumor cell interactions on tumor behavior has not been thoroughly investigated from the perspective of different stages of tumor progression. To address this question we assessed the influence of normal dermal fibroblasts on the growth of human melanoma cells obtained from different stages of tumor progression. We found that the *in vitro* growth of most (4 out of 5) melanoma cell lines derived from early-stage radial growth phase or vertical growth phase metastatically incompetent primary lesions is repressed by coculture with normal dermal fibroblasts, suggesting that negative homeostatic growth controls are still operative on melanoma cells from early stages of disease. On the other hand, 9 out of 11 melanoma cell lines derived from advanced metastatically competent vertical growth phase primary lesions, or from distant metastases, were found to be consistently *stimulated* to grow in the presence of dermal fibroblasts. Evidence was obtained to show that this discriminatory fibroblastic influence is mediated by soluble inhibitory and stimulatory growth factor(s). Taken together, these results indicate that fibroblast-derived signals can have antithetical growth effects on metastatic versus metastatically incompetent tumor subpopulations. This resultant conversion in responsiveness to host tissue environmental factors may confer upon small numbers of metastatically competent cells a growth advantage, allowing them to escape local growth constraints both in the primary tumor site and at distant ectopic tissue sites.**

Organ homeostasis is in part controlled by complex cellular interactions which help regulate cell proliferation and differentiation (1). The uncontrolled proliferation of cancer cells associated with the development of three-dimensional tumor masses may be the consequence of an escape from these normal homeostatic growth controls. In some cases the extent of this escape may be minimal or negligible, whereas in others it may be more profound. Thus, there have been a number of reports which have shown that the proliferation of neoplastic cells can be negatively regulated by the presence of adjacent normal cells (2–6). The enhancing effect of "removal" of adjacent normal cells on development of early neoplasia is probably also illustrated by *in vivo* models of the sequential analysis of cancer development, especially liver and skin cancers (7). This is especially apparent when "initiated" cells present in small preneoplastic nodules are confronted with various xenobiotic tumor-promoting agents, which selectively damage or destroy excess normal adjacent cells, thereby allowing the outgrowth of the "resistant," initiated preneoplastic cells (7).

Despite such normal cell growth controls, tumor cells can ultimately expand to form solid tumors and do so more aggressively with time in that they eventually infiltrate underlying tissues, gain access to the vasculature, and colonize new "ectopic" organ sites. This ability to grow aggressively in various tissue environments suggests that subpopulations of tumor cells exist which are relatively refractory to the growth repression exerted by adjacent normal cells. Such tumor cells may acquire "autonomous" growth properties (8–11), or, alternatively, may actually be *stimulated* to grow by the presence of such normal cells and/or their products.

We have chosen the human melanoma system to investigate the hypothesis that tumor cells acquire increasing degrees of resistance to the inhibitory homeostatic growth influences exerted by adjacent normal cells with more advanced (i.e., metastatically competent) stages of tumor progression and can actually be stimulated to grow at these final phases. Our choice of studying human melanoma was dictated in part by the clinical behavior and history of this type of cancer. Thus the majority of melanomas go through a series of well-defined stages of preneoplastic and neoplastic progression, a characteristic that allows for the derivation of cell lines from different and discrete stages of tumor progression (8). Melanomas can develop in a sequential, evolutionary, manner in which the epidermally or dermally confined "radial growth phase" (RGP) primary melanomas—which are not metastatically competent—eventually progress to the so-called "vertical growth phase" (VGP). Depending on the thickness of the VGP primary melanomas, they have a low or high probability of being metastatically competent—i.e., of being "malignant" tumors (8). "Early" VGP lesions are those less than 0.76 mm in thickness, and over 90% of melanoma patients have no evidence of recurrent disease after removal of such tumors. In contrast, thicker, more advanced lesions carry a much worse prognosis (8). In this regard, Herlyn and colleagues have succeeded in establishing a number of melanoma cell lines obtained from RGP, early primary VGP, advanced primary VGP, and distant metastatic lesions (8, 9, 12).

Because melanoma cells are in close proximity, or in direct contact, with dermal fibroblasts during the initial stages of tumor progression (i.e., after they penetrate the dermo-

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Abbreviations: RGP, radial growth phase; VGP, vertical growth phase; FCS, fetal calf serum.

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epidermal basement membrane), we elected to assess the *in vitro* influence of normal dermal fibroblasts on the proliferation of a panel of human melanoma cell lines derived from early, metastatically incompetent (i.e., RGP or thin VGP primary tumors), or advanced (i.e., thick VGP primary tumors or distant metastases) metastatically competent lesions. The results we obtained in the present study clearly demonstrate that the growth of melanoma cells isolated from early lesions is repressed by dermal fibroblasts, whereas metastatically competent melanoma cells are resistant, or, more commonly, their growth is actually *stimulated* in the presence of these fibroblasts. As such, our results provide another dimension to the possible relevance of tumor cell–host stromal interactions to tumor growth *in vivo*.

MATERIALS AND METHODS

Cell Lines. Melanoma cell cultures were maintained in RPMI 1640 medium (GIBCO) with 5% fetal calf serum (FCS) (GIBCO). WM35, WM1650, WM902B, WM793, WM1205, WM1341B, WM451, WM9, WM1361A, WM983A, and WM983B were established by M. Herlyn *et al.* (8). SKMEL28 was purchased from the American Type Culture Collection. MW122 and WM75C were provided by J. Jambrosic (University of Toronto), and UACC903 was supplied by J. Trent (University of Michigan, Ann Arbor). MeWo has been extensively described elsewhere (13). The clinical and pathological characteristics of the melanoma lesions from which the cell lines were derived are reported in *Results* and elsewhere (8, 9, 12).

Normal dermal and tumor-derived fibroblasts were obtained by standard culture explants from newborn foreskins and from melanoma chunks, respectively. Fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO) supplemented with 10% FCS and were used for experiments between passage 3 and 15. Umbilical vein endothelial cells were harvested according to a technique described elsewhere (14). Bovine capillary microvascular cells, provided by J. Folkman (Harvard University, Boston), were obtained from adrenal gland. Endothelial cells were cultured on gelatin-coated plates in DMEM supplemented with 10% FCS and endothelial mitogen (Biomedical Technologies, Stoughton, MA) at 120 μg/ml in a 10% CO_2 atmosphere. Murine adipocyte cultures resulted from the *in vitro* differentiation induction by insulin of BALB/c-3T3 (clone A31T6) preadipocytes generously provided by T. O'Brien at 1 μg/ml. Mink lung epithelial cells, Daudi cells, and NIH-3T3 cells were purchased from the American Type Culture Collection. We also obtained several nonneoplastic human skin fibroblast cell populations from the American Type Culture Collection. They were all originally derived from skin biopsy materials. These cell lines were CRL-1336, obtained from a patient with Cockayne syndrome; CRL-1244, obtained from a patient with dermatomyositis; CR-1246, obtained from a cyclops syndrome patient; and CRL-1131, obtained from an Ehlers–Danlos syndrome patient. All these cell lines have a limited life-span (15–35 serial subcultivations) and were used at relatively early subcultivation passage, after being received from the American Type Culture Collection. They were used in the coculture experiments with human melanoma cells, as described below.

Cocultures of Normal Cells and Melanoma Cells. Monolayer cultures of 2.5×10^4 fibroblasts were seeded in 96-well plates (Costar) in DMEM with 5% FCS. Twenty-four hours later, the fibroblasts (52,000 cells per cm^2) were highly packed on each other and were either γ-irradiated (4000 rads; 1 rad = 0.01 Gy) or not. The fibroblast medium was removed and the cell layers were carefully washed twice with MCDB 153-L15 (Irvine) plus 2% FCS. Then 5×10^3 melanoma cells were plated on the fibroblast layers in 100 μl of MCDB 153-L15 plus 2% FCS. After 48-hr incubation, the cocultures were pulsed for 6 hr with 2 μCi of [^{3}H]thymidine (Amersham; specific activity 8.3 mCi/mg; 1 Ci = 37 GBq). After cell trypsinization the DNA was harvested onto filter mats (Skatron, Sterling, VA) and [^{3}H]thymidine incorporation was measured with a β-counter (LKB). In the case of coculture with adipocytes, 2.5×10^4 preadipocytes were deposited into 96-well plates and once confluent they were induced to differentiate with insulin at 1 μg/ml. Two to 3 weeks later, at least two-thirds of the preadipocytes had differentiated into adipocytes and cocultures with melanoma cells were then undertaken. Each coculture was done in triplicate.

Conditioned Media. Conditioned media from confluent fibroblasts ($1.0–1.5 \times 10^6$ fibroblasts per 8.8-cm diameter Nunc dish) and from cocultures were harvested after 48-hr culture in 7 ml of MCDB 153-L15 plus 2% FCS. The supernatants were centrifuged at $1000 \times g$ for 10 min to remove cellular debris and concentrated 10 times by membrane ultrafiltration (Amicon YMT membrane; cut-off molecular weight 10,000) in a centrifuge Concentrator-10. The concentrated media were diluted 1:1 with fresh MCDB 153-L15 medium plus 2% FCS and incubated for 48 hr with 5×10^3 melanoma cells seeded in 96-well plates. The cultures were pulsed with [^{3}H]thymidine and harvested as described above.

Monolayer Cocultures Without Cell Contact. Melanoma–fibroblast cocultures without contact were grown in Sterilin (Teddington, Middlesex, U.K.) Cell-Cult 4-well plates. Because the well sides are very low, the entire plate can be flooded with medium and diffusible factors can then circulate between the wells. Two wells of a 4-well plate were seeded with 2×10^4 fibroblasts in 100 μl of MCDB 153-L15 plus 2% FCS. Fibroblast confluency was attained in 48 hr. Then 2×10^3 melanoma cells were seeded into the two remaining wells in 100 μl of MCDB 153-L15 plus 2% FCS medium. The next day, the entire plate was covered with the medium. At 3 and 5 days of incubation, the melanoma cells cultured alone or in presence of fibroblasts were harvested and counted in quadruplicate by using a Coulter Counter.

RESULTS

Effect of Normal Human Dermal Fibroblasts on [^{3}H]Thymidine Uptake by Human Melanoma Cells. To analyze the influence of fibroblasts on melanoma cell proliferation, we initially measured [^{3}H]thymidine incorporation by human melanoma cells derived from early and late lesions when cultured on a confluent monolayer of fibroblasts in comparison to culture on plastic alone. Early lesions include RGP melanomas (WM35 and WM1650 cell lines) and "thin" VGP melanomas from patients who were free of metastatic spread as long as 68 months after removal of the primary tumor (WM902B, WM1341B, and WM793 cell lines). Late or advanced lesions included VGP primary melanomas obtained from patients with concomitant distant metastases at the time of tumor removal (WM1361A, WM983A, and WM122 cell lines) and cell lines from organ or lymph node metastases (MeWo, SKMEL28, WM451, WM983B, UACC903, and WM75 cell lines). We found that four cell lines derived from early stage (thin) primary lesions were inhibited in their [^{3}H]thymidine incorporation by 1.4- to 3.3-fold when grown in the presence of dermal fibroblasts (Fig. 1*A*). The nonmetastatic VGP-derived WM793 was the only cell line in this category that was not found to be inhibited. Under identical culture conditions, the [^{3}H]thymidine incorporation of 9 out of 11 melanoma cell lines derived from advanced lesions was significantly enhanced, by 1.6- to 2.6-fold, in the presence of dermal fibroblasts. Two such cell lines (WM122 and WM75C) were found to be inhibited (Fig. 1*B*). Similar results were obtained whether or not the dermal fibroblast layers were γ-irradiated. To study this phenomenon further, genetically related cell lines obtained from early and late lesions should be tested in the coculture system described. Because over

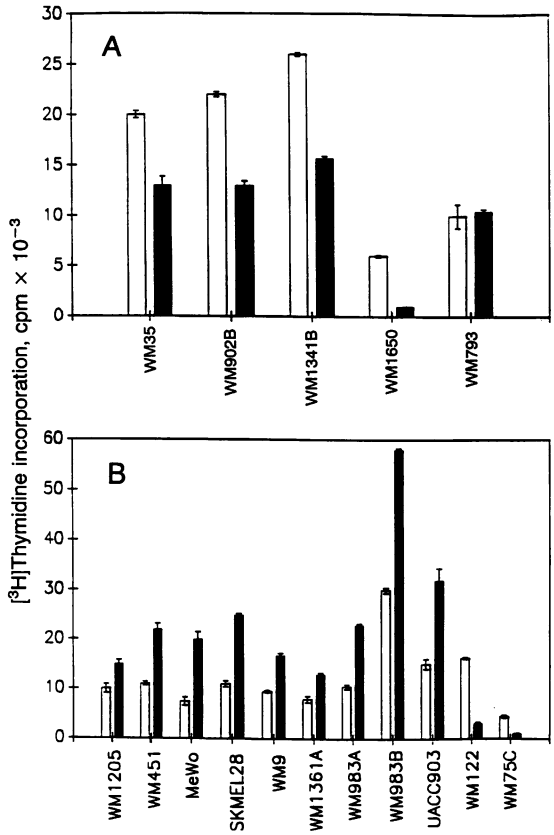

FIG. 1. Influence of γ-irradiated human dermal fibroblasts on the [^{3}H]thymidine incorporation of human melanoma cells derived from early (*A*) and advanced (*B*) lesions. □, [^{3}H]Thymidine incorporation of melanoma cells on plastic; ■, [^{3}H]thymidine incorporation of melanoma cell–fibroblast cultures with the control irradiated fibroblast incorporation (452 ± 27 cpm) subtracted. The reported means of [^{3}H]thymidine incorporation and the standard errors are calculated from triplicates.

95% of patients having early melanoma lesions are cured by surgery, such related or "paired" cell lines are not available. To circumvent this problem, the early VGP-derived WM793 cell line was grown in *nude* mice as a solid tumor after subcutaneous injection of the cells. This resulted in some lung metastases after several passages; one such tumor nodule was established in tissue culture and was designated as WM1205. It was found to be metastatic upon subcutaneous injection into *nude* mouse recipients (M.H., unpublished observations). We observed that whereas the thymidine incorporation of the parental WM793 cells was not affected by dermal fibroblasts, the incorporation of the metastasis-derived WM1205 cells was stimulated (Fig. 1). We also tested four different adult human fibroblast cell strains obtained from the American Type Culture Collection, as described in *Materials and Methods*. These cell lines were CRL-1336, CRL-1346, CRL-1244, and CRL-1131. They behaved in an identical manner to the normal dermal fibroblasts we used in the experiments described above, both quantitatively and qualitatively.

Specificity of the Fibroblastic Influence on Melanoma Growth. Fibroblasts from tumors have been reported to display abnormal phenotypic characteristics and manifest fetal-fibroblast-like characteristics (15). To assess the influence of fibroblasts infiltrating melanomas on melanoma cell [^{3}H]thymidine incorporation, we performed a coculture assay with the MW35 (RGP) and MeWo (metastasis) cell lines involving fibroblasts obtained from a dysplastic nevus, a RGP lesion, and a distant metastasis. Fig. 2 shows that the growth-suppressive or -stimulatory effects are similar for newborn-derived fibroblasts and adult fibroblasts obtained from benign or malignant melanomas.

We then asked if the fibroblast effect on melanoma cell thymidine incorporation was peculiar to dermal fibroblasts or if it can be detected when any type of normal cell population is used. Coculture experiments in which fibroblasts were replaced with endothelial cells or with adipocytes were therefore undertaken. Endothelial cells were obtained from the human umbilical vein or from the bovine adrenal gland. The adipocytes were induced to differentiate *in vitro* from murine BALB/c-3T3 preadipocytes. We found that endothelial cells do not modulate significantly the [^{3}H]thymidine incorporation by human melanoma cells, whereas murine adipocytes were inhibitory for all melanoma cell lines regardless of their relative malignant status (Fig. 3). These results demonstrate that the antithetical fibroblastic effect on metastatic versus nonmetastatic melanomas may not be reproduced with other types of normal cell populations and raises the possibility that the results may be specific to the fibroblastic lineage, possibly to fibroblasts of dermal origin.

Evidence for (a) Soluble Factor(s) Mediating the Fibroblastic Effect on Melanoma Cells. Several mechanisms involving gap-junction communications, extracellular matrix components, and/or soluble secreted factors may contribute to the fibroblastic effect on melanoma cell [^{3}H]thymidine incorporation. Indeed, there is evidence from some systems that the suppressive effect of adjacent normal cells on small numbers of transformed neoplastic cells is mediated by intimate cell contact via gap junctions (16). In this regard, no effect on melanoma cell [^{3}H]thymidine incorporation could be obtained with extracellular matrix components laid down by fibroblasts or by the coculture of fibroblasts and melanoma cells (data not shown). Similarly, purified collagen type I, collagen type IV, and laminin layer did not significantly modulate [^{3}H]thymidine incorporation by human melanoma cells in tissue culture (data not shown).

The effect of 10-fold concentrated supernatant from 48-hr fibroblast cultures diluted 1:1 with fresh MCDB 153-L15 supplemented with 2% FCS was tested in a [^{3}H]thymidine incorporation assay on the nonmetastatic WM35 (RGP) and WM9 (metastasis) cell lines. Compared to control medium, the fibroblast concentrated medium reduced the [^{3}H]thymidine incorporation of WM35 cells by 5.6-fold, whereas it increased the [^{3}H]thymidine incorporation of WM9 cells by 1.5-fold (Fig. 4). No further inhibition or stimulation could be obtained with supernatant from cocultures of fibroblasts and

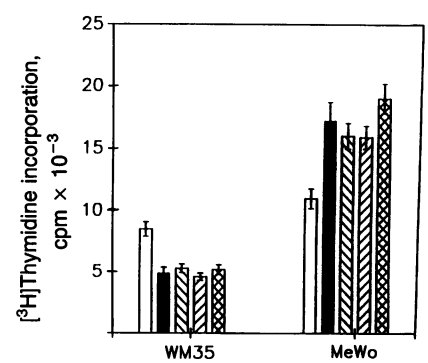

FIG. 2. Comparative effect of γ-irradiated normal, dysplastic-nevus-derived, or tumor-derived fibroblasts on the [^{3}H]thymidine incorporation of WM35 (RGP-derived) and MeWo (metastasis-derived) cells. Melanoma cell incorporation is shown on plastic (□), onnewborn-foreskin-derived fibroblasts (■), on dysplastic-nevus-derived fibroblasts (▨), on RGP-derived fibroblasts (▨), and on metastatic VGP-derived fibroblasts (▩). In each case, the [^{3}H]thymidine incorporation of the irradiated fibroblasts was subtracted from the coculture incorporation.

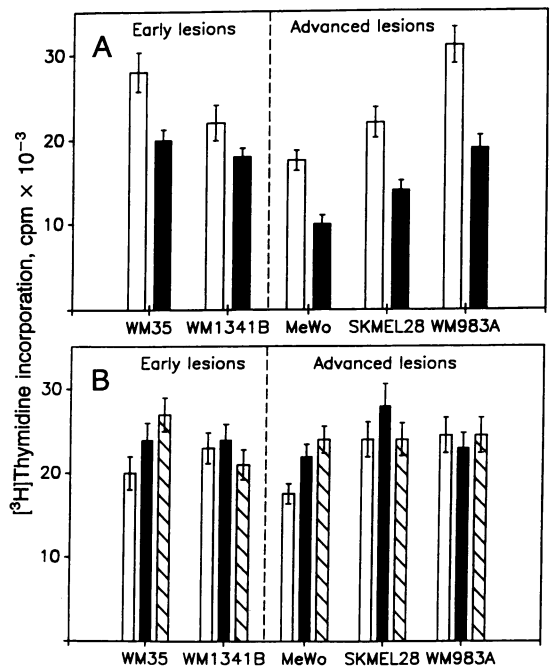

FIG. 3. Influence of γ-irradiated adipocytes (*A*) or endothelial cells (*B*) on human melanoma cell [^{3}H]thymidine incorporation. (*A*) Melanoma cell [^{3}H]thymidine incorporation on plastic (▭) and on differentiated murine adipocytes minus the control irradiated adipocyte incorporation (802 ± 123 cpm) (■). (*B*) Melanoma cell [^{3}H]thymidine incorporation on plastic (▭), on human umbilical-vein-derived endothelial cells minus the control irradiated endothelial cells (223 ± 89) (■), and on bovine adrenal-derived capillary cells minus the control irradiated endothelial cells (352 ± 101 cpm) (▨). The reported means of [^{3}H]thymidine incorporation and the standard errors are calculated from triplicates.

melanoma cells, indicating that reciprocal interactions between melanoma cells and fibroblasts are not required for the fibroblastic effect (Fig. 4). However, this does not rule out a certain degree of reciprocal interactions between melanoma cells and fibroblasts in the coculture situation that cannot be revealed in the "swapping" experiments. The stimulatory effect on WM9 cells became nondetectable at a 1:5 dilution and the inhibitory effect at 1:50 dilution (data not shown).

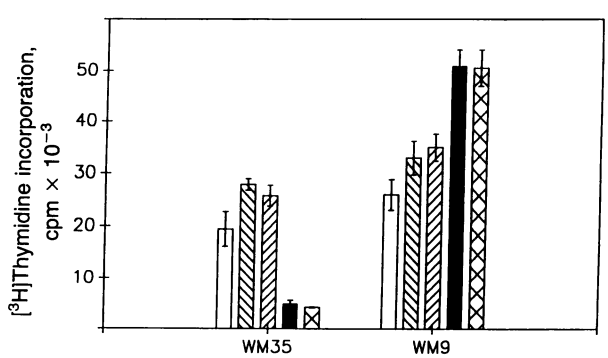

FIG. 4. Effect of conditioned media on the WM35 (RGP-derived) and WM9 (metastasis-derived) cell [^{3}H]thymidine incorporation. Melanoma cell [^{3}H]thymidine incorporation is shown in base medium (MCDB 153-L15 plus 2% FCS) (▭), in 1:1 diluted base medium and 10× concentrated base medium (▨), in 1:1 diluted base medium and 10× concentrated melanoma cell supernatant (▨), in 1:1 diluted base medium and 10× concentrated fibroblast supernatant (■), and in 1:1 diluted base medium and 10× concentrated fibroblast–melanoma cell coculture supernatant (▩). The reported means of [^{3}H]thymidine incorporation and the standard errors are calculated from triplicates.

To show that the modulations of [^{3}H]thymidine incorporation reflected relative cellular proliferation, we cocultured melanoma cells and fibroblasts in separated wells of a Sterilin Cell-Cult plate, where the two cell types share the same medium but are not in contact. Melanoma cells were counted after 3 and 5 days of coculture before they had reached confluency. After 5 days, the growth of WM9 cells (metastatic) was stimulated 1.6-fold, whereas the growth of nonmetastatic WM35 cells (RGP) was inhibited by 3.5-fold (Fig. 5). In the conditioned medium experiment described above, we observed a loss of the fibroblast stimulatory effect on WM9 cells when the fibroblast-conditioned medium had been diluted 5-fold, which corresponds to 2×10^4 fibroblast equivalents/100 μl. In the Cell-Cult plate assay, the fibroblast production can be estimated as 10^4 fibroblast cell equivalents/100 μl, which had been found to be stimulatory for WM9 cells. It is possible that the putative growth factor, or one of the putative growth factors involved, is unstable and that some activity may be lost during the centrifugation or concentration steps or during further incubation, with no self-renewal; in addition, the growth factor(s) may have some affinity for the membrane filter of the concentrator.

DISCUSSION

During embryogenesis, premelanocytes migrate from the neural crest and differentiate into melanocytes as a result of complex interactions with the dermal mesoderm and the epidermal ectoderm (17). In adult skin, dermal fibroblasts also appear to control or affect melanocyte biology, and the nature of this control is thought to be altered during melanocyte tumorigenesis and melanoma tumor progression (1). This report provides evidence that human dermal fibroblasts repress the *in vitro* growth of melanoma cells derived from early benign (nonmetastatic) lesions and that this inhibitory effect is "converted" to a growth-stimulatory one for most cell lines established from more advanced malignant melanomas. Thus, we found that newborn dermal fibroblasts and adult fibroblasts obtained from a preneoplastic dysplastic nevus, an RGP, and a melanoma metastasis, as well as four fibroblast cell "strains," were similarly discriminatory for poorly and highly malignant human melanoma-derived cell lines. Because we found that murine dermal fibroblasts had an effect on melanoma cell [^{3}H]thymidine incorporation similar to that of human dermal fibroblasts (data not shown), the nonregulatory effect of murine adipocytes and the nondiscriminatory effect of bovine endothelial cells on human melanoma cells from early and late lesions are unlikely to be explained by their cross-species origin. The pattern of the fibroblastic influence on melanoma cell growth may also be specific for the fibroblast lineage. Fibroblasts constitute a

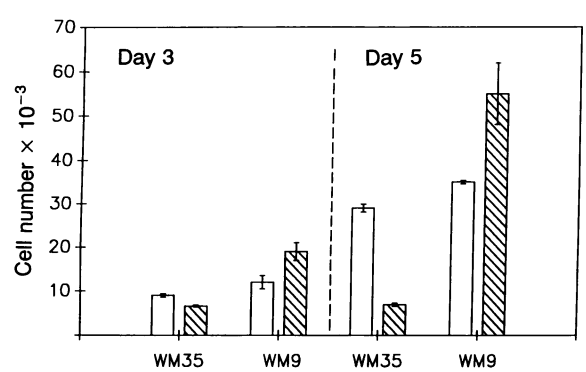

FIG. 5. Coculture of human dermal fibroblasts and melanoma cells in separated wells of a 4-well plate. ▭, Melanoma cell number in absence of dermal fibroblasts; ▨, melanoma cell number in presence of dermal fibroblasts. The reported mean of cell number and the standard errors are calculated from quadruplicates.

poorly defined cellular category, and their morphology, growth requirements, and response to growth factors may vary with their tissue of origin (18). It is therefore possible that the fibroblastic effect on melanoma cell growth is limited to fibroblasts of dermal origin or even to a particular dermal subpopulation.

Cocultures of melanoma cells and fibroblasts *without* cellular contact revealed the evidence for involvement of soluble growth inhibitor(s) or stimulator(s) as mediator(s) of the fibroblastic effects observed. A number of proteins have recently been characterized that possess potent growth-inhibitory properties towards a variety of cell types in culture. Among those proteins, the family of transforming growth factor β (TGFβ) (18) and interferon β (19) are potentially secreted by fibroblasts. In addition, two growth-inhibitory molecules have been purified from medium conditioned by dense cultures of immortalized mouse fibroblasts—i.e., the IDF45 (20) and a 13-kDa molecule (21) that is structurally related to a mammary-derived growth inhibitor (22). These inhibitory molecules are all possible candidates as mediators of the fibroblastic inhibition of melanoma cell proliferation. So too is interleukin 6 (IL-6), which was recently shown to be an inhibitor of human melanocyte proliferation (23). It is also known to be secreted by fibroblasts (24). The commercial availability of neutralizing antibodies against TGFβ and interferon β allowed us to rule out the participation of these two growth inhibitors (unpublished observations). Similarly, neutralizing antibodies against growth factors known to be stimulatory for melanomas (12) were used to determine the possible involvement of such growth factors in the enhanced growth of cells from advanced malignant melanoma lesions by dermal fibroblasts. None of the growth factors tested (transforming growth factor α, epidermal growth factor, basic fibroblast growth factor, or insulin-like growth factor 1) could be identified as being mediators of the stimulatory fibroblastic effect (I.C., S.M., and R.S.K., unpublished observations). Ion-exchange and gel-filtration chromatography of dermal-fibroblast-conditioned media has revealed preliminary evidence that the inhibitor is a protein that is separable from the stimulatory activity (C. Lu and R.S.K., unpublished observations).

There are previous reports that tumor progression can be accompanied by changes in response to or release of various growth factors by tumor cells. For example, transforming growth factor β inhibits immortalized 10T½ mouse fibroblasts but stimulates *ras*-transformed and metastatic 10T½ fibroblasts (11); IDF45 inhibits reversibly the growth and DNA synthesis in chicken embryo fibroblasts (CEF), but little growth inhibitory effect was observed with CEF cells transformed by v-*src* oncogene (20); bladder-derived fibroblasts stimulate the proliferation of the poorly differentiated high-grade invasive bladder-carcinoma-derived EJ cell line but not the growth of the well-differentiated bladder-carcinoma-derived RT4 cell line (25). Therefore, the conversion of the fibroblastic effect from growth inhibition to growth stimulation during melanoma tumor progression may not be restricted to melanoma, but may be more generic in nature. Similarly, normal mammary cells suppress the growth of preneoplastic nodules but promote the growth of more advanced mammary carcinoma lesions (26). Such effects may be a major contributing factor to the "growth-dominant" phenotype of metastatic or metastatically competent tumor cell subpopulations in certain types of neoplasms and thus contribute to their inexorable overgrowth ("clonal dominance") at the primary tumor site (27), as well as their ability to grow in foreign or ectopic organ sites as distant metastases.

Given their widespread distribution in connective soft tissues adjacent to various epithelial compartments throughout the body, fibroblasts may act as a potential "cellular fertilizer" to help nourish the growth of nascent micrometastases (assuming the fibroblast effects are not tissue specific). In this respect it is also important to note that there is often a prominent, "reactive" stromal fibroblastic hyperplasia within and around growing tumors (28, 29). Indeed, there is also evidence that metastatic cancer cell subpopulations may help induce such "activated" stromal fibroblasts (28). This may lead to a reciprocal and expanding paracrine interaction between adjacent fibroblasts and malignant tumor cells, thereby facilitating the growth of both cell populations, and hence tumor progression as well as metastasis.

Our results provide an interesting paradigm to illustrate the concept that the manner in which adjacent normal cells affect tumor cell growth can be profoundly altered by the relative malignant status of the tumor cell population. Thus, while our results confirm the idea that an excess of nontumorigenic cells may restrict the growth of an "interspersed" tumorigenic minority subpopulation (30), they also show that this growth-restraining process may be converted to a growth-facilitating one by highly progressed (advanced) metastatically competent tumor cells.

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